

Accession No. 405399-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Verbenone. Shaughnessey Number: 128986.
2. **TEST MATERIAL:** Verbenone; 100% active ingredient; a clear liquid.
3. **STUDY TYPE:** Acute Toxicity Test for Freshwater Fish.
Species Tested: Bluegill (Lepomis macrochirus).
4. **CITATION:** Surprenant, D.C. 1988. Static Acute Toxicity of Verbenone to Bluegill (Lepomis macrochirus). Submitted by Phero Tech, Inc., Vancouver, B.C., Canada. Study performed by Springborn Life Sciences, Inc., Wareham, MA. Laboratory Report No. 88-2-2634. EPA Accession No. 405399-04.

5. **REVIEWED BY:**

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Signature: *Michael L. Whitten*

Date: 8-10-89

6. **APPROVED BY:**

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Signature: *Isabel C. Johnson*

Date: August 11, 1989

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7. **CONCLUSIONS:** This study appears scientifically sound but does not fulfill the requirements for a 96-hour static acute toxicity study. Based upon nominal concentrations, the 96-hour LC50 of Verbenone to bluegill sunfish was 206 mg/L. This value classifies Verbenone as practically non-toxic to bluegill sunfish. The NOEC was determined to be 130 mg/L. Since the test chemical was partially insoluble, the actual concentrations to which the fish were exposed is unknown.
8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bluegill sunfish (Lepomis macrochirus) were obtained from a commercial fish supplier in Missouri. The fish were held in a 500 L fiberglass tank containing well water for a minimum of 14 days at 13-21°C. During the ten days prior to testing the temperature in the holding tank ranged from 19°C to 21°C. The fish were fed a dry commercial pelleted food, ad libitum, daily except during the 48 hours prior to testing. There was no mortality in the test fish population during the 48-hour period prior to testing. The fish used for the experiment had a mean wet weight of 0.69 g with a range of 0.39 to 1.31 g and a mean total length of 40 mm with a range of 34 to 49 mm.

B. **Test System:** The test was conducted in 18.9 L glass aquaria which contained 15 L of test solution. The test solution depth was 18.4 cm with a surface area of 819 cm². Test solutions were prepared by directly adding the appropriate quantity of Verbenone to 15 L of dilution water in each test aquarium and then mixed with a laboratory stirrer. A control aquarium was established and maintained under the same conditions as the test aquaria but contained no Verbenone.

The dilution water was soft water reconstituted from deionized water. The dilution water had a total hardness of 48 mg/L as CaCO₃, a total alkalinity of 33 mg/L as CaCO₃, a pH of 7.8, and a specific conductance of 300 umhos/cm.

All test solution temperatures were controlled by a system designed to maintain temperatures at 22 ± 1°C. Aeration was initiated in all aquaria at 48 hours and was continued throughout the exposure period. A photoperiod of 16 hours of light and 8 hours of darkness was provided each day.

C. **Dosage:** 96-hour acute static test.

D. **Design:** A control and five nominal concentrations of 78, 130, 220, 360, and 600 mg/L were tested. Ten fish selected impartially from the holding tank were placed

in each test aquarium within 15 minutes after the test solutions had been prepared. The resulting test organism loading was 0.46 g of biomass per liter of test solution. Fish were not fed during the test.

All aquaria were observed at 0, 24, 48, 72, and 96 hours of exposure for mortality and abnormal effects. Dissolved oxygen, pH, and temperature were measured in all aquaria at each 24-hour interval.

E. **Statistics:** The LC50 was determined by a computerized calculation program (Peltier, 1985) for each 24-hour interval.

12. **REPORTED RESULTS:** The nominal test concentrations and corresponding cumulative percent mortalities are presented in Table 1 (attached). At test initiation a precipitate was observed on the surface of the 78 and 130 mg/L test solutions and a film was present on the surface of the 220, 360 and 600 mg/L solutions. Throughout the remainder of the exposure period a film of undissolved test material was present on the surface of all solutions containing Verbenone.

The 96-hour LC50 was estimated by nonlinear interpolation to be 210 mg/L with a 95% confidence interval calculated by binomial probability of 130 to 360 mg/L. Based on the results of this study, Verbenone is considered practically non-toxic to bluegill.

The dissolved oxygen concentrations and pH measured during the test are shown in Table 3 (attached). The dissolved oxygen concentration remained above the required 60% of saturation in all aquaria during the first 24 hours but ranged from 46% to 56% of saturation at 48 hours. Aeration was initiated at this point after which the dissolved oxygen concentration remained above 85% saturation in all aquaria. The temperature ranged from 20°C to 21°C during the exposure period.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author presented no conclusions other than various remarks reported in Section 12, above.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report. The director stated that the study followed "...all pertinent EPA Good Laboratory Practice regulations except in the case of

characterization and verification of the test substance identity."

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS

- A. Test Procedure: The test procedures were in accordance with recommended protocols with the following exceptions:

The SEP states that individual fish should weigh between 0.5 and 5 grams. Some fish used in this study weighed as low as 0.39 gram.

The SEP states that the test temperature for warmwater fish should be 17 or 22°C. During the toxicity test, the temperature ranged from 20 to 21°C.

The SEP states that if test material solubility problems are encountered (i.e., a precipitate is present), chemical analyses are required to verify test exposure concentrations. Test concentrations were not measured in this study, although precipitates and surface films were reported.

The SEP states that test solutions should be analyzed to determine the exact concentration of test material if the test solutions are aerated, since aeration may cause volatilization of the pesticide. In this study, aeration was initiated at 48 hours in all aquaria. As reported above, however, test concentrations were not measured.

- B. Statistical Analysis: Using EPA's Toxanal computer program, the LC50 was estimated (attached) by the binomial test to be 206 mg/L with a 95 percent confidence interval of 130 to 360 mg/L, which is practically the same as reported by the author (210 mg/L).

- C. Discussion/Results: At test initiation a precipitate was observed on the surface of the 78 and 130 mg/L test solutions and a film was present on the surface of the 220, 360 and 600 mg/L solutions. Throughout the remainder of the exposure period a film of undissolved test material was observed on the surface of all solutions containing Verbenone. The partial insolubility of the test chemical indicates that the concentrations of toxicant were less than the nominal values.

All mortalities occurred within 24 hours of test initiation. The 96-hour no-observed-effect concentration (NOEC) was 130 mg/L, based on the loss of equilibrium of fish in the 220 mg/L group. The 96-hour LC50 of 206 mg/L (based upon nominal concentrations) classifies Verbenone as practically non-toxic to bluegill sunfish (Lepomis macrochirus).

The test procedures appear to be scientifically valid, but since the test chemical was partially insoluble and the test solutions were aerated, the actual concentrations to which the fish were exposed is unknown.

D. Adequacy of the Study:

- (1) **Classification:** Supplemental
- (2) **Rationale:** Problems with solubility preclude a proper determination of toxicant concentrations.
- (3) **Repairability:** Yes, if the registrant can show that the concentration of Verbenone was not seriously less than the nominal concentrations. An LC50 of greater than 100 mg/L would still classify Verbenone as practically non-toxic.

15. COMPLETION OF ONE-LINER: Yes, 7-31-89.

Table 1. Concentrations tested, corresponding cumulative mortalities and observations made during the 96 hour static exposure of bluegill (Lepomis macrochirus) to Verbenone.

Nominal Concentration (mg/L)	Cumulative Mortality (%)			
	24-hour	48-hour	72-hour	96-hour
Control	0	0	0	0
78	0 ^a	0 ^a	0 ^a	0 ^a
130	0 ^a	0 ^a	0 ^a	0 ^a
220	60 ^{ab}	60 ^{ab}	60 ^{ab}	60 ^{ab}
360	100 ^a	100 ^a	100 ^a	100 ^a
600	100 ^a	100 ^a	100 ^a	100 ^a

^aA film was present on the surface of the test solution.

^bSeveral of the surviving fish exhibited a complete loss of equilibrium and were on the bottom of the test solution.

Table 3. pH and dissolved oxygen concentration measured during the 96-hour static exposure of bluegill (Lepomis macrochirus) to Verbenone.

Nominal Concentration (mg/L)	0-hour	24-hour	48-hour	72-hour	96-hour
<u>pH</u>					
Control	7.7	7.3	7.3	7.7	7.8
78	7.8	7.3	7.3	7.7	7.7
130	7.8	7.3	7.3	7.6	7.7
220	7.8	7.3	7.2	7.7	7.8
360	7.8	7.3	--- ^a	---	---
600	7.8	7.3	---	---	---
<u>Dissolved Oxygen, mg/L</u> (% Saturation)					
Control	9.6 (105)	6.8 (76)	4.5 (50)	8.6 (96)	7.8 (87)
78	9.6 (105)	6.8 (76)	4.1 (46)	8.4 (94)	7.7 (86)
130	9.6 (105)	6.4 (72)	4.3 (48)	8.6 (96)	7.6 (85)
220	9.6 (105)	7.2 (81)	5.0 (56)	8.6 (96)	7.8 (87)
360	9.6 (105)	7.7 (86)	---	---	---
600	9.6 (105)	7.7 (86)	---	---	---

^aMeasurements not required due to 100% mortality at the previous 24 hour observation interval.

WHITTEN VERBENONE LEPOMIS MACROCHIRUS 07-31-89

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
600	10	10	100	9.765625E-02
360	10	10	100	9.765625E-02
220	10	6	60.00001	37.69531
130	10	0	0	9.765625E-02
78	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 130 AND 360 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 205.8026

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

Casey No. 128986Chemical Name Verbenone Chemical Class _____Page 1 of 1

ay/Species/Lab/ Accession	Chemical X a.l.	Results	Reviewer/ Date	Valid Sta
14-Day Single Dose Oral LD ₅₀		LD ₅₀ = mg/kg (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Age(Days) = Sex = 14-Day Dose Level mg/kg/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				
14-Day Single Dose Oral LD ₅₀		LD ₅₀ = mg/kg. (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Age(Days) = Sex = 14-Day Dose Level mg/kg/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				
8-Day Dietary LC ₅₀		LC ₅₀ = ppm (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Age(Days) = Sex = 8-Day Dose Level ppm/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				
8-Day Dietary LC ₅₀		LC ₅₀ = ppm (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Age(Days) = Sex = 8-Day Dose Level ppm/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				
48-Hour LC ₅₀		LC ₅₀ = pp (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Sol. Contr. Mort.(X) = Temperature = 48-Hour Dose Level pp/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				
96-Hour LC ₅₀		LC ₅₀ = 206 ppm (<u>95% C.L.</u>) Contr. Mort.(X) = 0 Slope = N/A # Animals/Level = 10 Sol. Contr. Mort.(X) = N/A Temp. = 20°C 96-Hour Dose Level pp/(X Mortality) 78 (0 , 130 (0 , 220 (60 , 360 (100 , 600 (100) Comments: BASED ON NOMINAL CONCENTRATIONS		
Species <u>Lepomis macrochirus</u>				
Lab <u>Springborn Life Sciences</u>				
Acc. <u>405399-04</u>				
96-Hour LC ₅₀		LC ₅₀ = pp (<u>95% C.L.</u>) Contr. Mort.(X) = Slope = # Animals/Level = Sol. Contr. Mort.(X) = Temp. = 96-Hour Dose Level pp/(X Mortality) (, , , , , , ,) Comments:		
Species				
Lab				
Acc.				

M. WHITTEN 7-31-89
Supplemental